THE RELATION OF REDUCTION OF LEUKOCYTES AND PLATELETS TO COMPLEMENTING POWER OF SERUM

E. E. ECKER; B. S. KLINE, AND A. DECALUWE

From the Department of Pathology, Western Reserve University, Cleveland, Ohio

The rôle of the leukocytes in the production of complement has been a matter of considerable study and dispute. The greater bulk of work on the subject was done when no clear differentiation had been made between amboceptor and complement, and by various methods of making extracts of white blood corpuscles. In vivo experiments have not received sufficient consideration, and it is with this point in view that we have attempted to reduce the number of the circulating leukocytes to ascertain whether or not a parallel reduction of complement could be demonstrated.

Historical Survey.—The hypothesis that complement might originate in the leukocytes was first advanced by Metchnikoff.[1] The original observation of Metchnikoff, however, was limited to the bactericidal substances contained in the polymorphonuclears. Following the work of Tarassewitch,[2] Shibayama,[3] and of Klein,[4] who showed that extracts of spleen, pancreas, omentum and lymph glands were endowed with hemolytic properties, Metchnikoff conceived the idea that the bacteriolytic complement was contained in the polymorphonuclear leukocytes (microcytase) and that the hemolytic complement was a different body and contained in macrophages (macrocytase). Metchnikoff received the support of Wassermann,[5] Ascoli and Riva,[6] Hahn[7] and others. It must be emphasized that in much of the earlier work insufficient attention was paid to carefully separating cells from serum. Some investigators (Wassermann, Ascoli and Riva) used an anticomplement produced by injecting washed leukocytes in rabbits, the resulting immune serum having an inhibiting action on hemolysis. However, similar observations were made by Donath and Landsteiner[8] who used red blood corpuscles, lymphnodes, milk, etc. The phenomenon, therefore, was not specific and not due to an anticomplement, as originally conceived. Prior to the latter studies, Schattenfroh[9] and Moxter[10] had shown that the bactericidal substances of polymorphonuclear leukocytes did not act as complement in hemolysis, and following their work Lambotte

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1 Ann. l'Inst. Pasteur, 1899, 13, p. 737.
2 Ibid., 1902, 16, p. 127.
3 Centralbl. f. Bakteriol., I, O., 1902, 30, p. 760.
4 Wien. klin. Wchnschr., 1901, 14, p. 1309.
5 Ztschr. f. Hyg. u. Infektionskr., 1901, 37, p. 173.
6 München. med. Wchnschr., 1901, 48, p. 1343.
7 Arch. f. Hyg., 1895, 25, p. 105.
8 Ztschr. f. Hyg. u. Infektionskr., 1903, 43, p. 552.
9 Arch. f. Hyg., 1897, 31, p. 1.
10 Deutsch. med. Wchnschr., 1899, 25, p. 687.

and Stiennon,[11] Petterson,[12] Schneider,[13] Gruber,[14] Neufeld,[15] Kling,[16] Zinsser,[17] Dick,[18] and recently Gengou[19] have made similar demonstrations. From these studies we have learned that leukocyte extracts resist a temperature of 60 C., a fact that in itself separates them from complement. Gengou, furthermore, found that these extracts do not contain mid- or end-piece of the complement. So far we have been able to find, Lippmann and Plesch[20] are the only investigators who studied the problem in vivo. They found that the regeneration of complement in guinea-pigs occurs after the destruction of the bone marrow and the leukocytes of the blood by injections of large quantities of thorium X. In our series of experiments we have used benzene and in counting the leukocytes also counted the blood platelets to establish their relation to complements.

Method.—Equal parts of benzene and olive oil were mixed and injected subcutaneously in doses of 1 c c benzene per kilo of animal. Rabbits were selected because of their known susceptibility to benzene and because their serum contains a fair amount of complement. Guinea-pigs were found to be resistant to benzene; double the amount necessary for the rabbit fails to reduce the circulating leukocytes of the guinea-pig. The benzene, furthermore, produces extensive necrosis in the guinea-pig and secondary infection readily occurs. Following the injections of the benzene olive oil mixture into rabbits, the site of injection was carefully massaged to allow a wide area of absorption. Three or 4 injections sufficed to reduce the circulating leukocytes to a figure below 1,000 per c. mm. In the first series of experiments the platelet counts were made by allowing blood to flow from the marginal vein through a needle into a syringe barrel containing 2% sodium citrate. The piston was then introduced and the barrel inverted a few times. Smears were made on glass slides and stained with Wright's stain. Comparative counts of platelets and red cells were then made. At least 40 fields covering the entire smear were counted and a separate count of erythrocytes made by the usual pipet counting chamber method. In the second series of experiments a brilliant cresyl blue citrate solution was used, and direct counts of platelets were made. The white count was made as usual.

The complement was titrated by diluting the serum with equal parts of Ringer-Tyrode's solution. Four units of amboceptor were used and

[11] Centralbl. f. Bakteriol., I, O., 1906, 40, p. 224.
[12] Ibid., 1905, 39, p. 423; 1908, 46, p. 405.
[13] Arch. f. Hyg., 1909, 70, p. 40.
[14] Cited by Sachs in Kraus and Levaditi, Handbuch der Technik. u. Methodik. der Immunitätsforschung.
[15] Arb. a. d. Kais. Gesundh. Anat., 1908, 28, p. 198.
[16] Ztschr. f. Immunitätsf. 1910, 7, p. 1.
[17] Infection and Resistance, 1918.
[18] Jour. Infect. Dis., 1913, 12, p. 111.
[19] Ann. l'Inst. Pasteur, 1921, 35, p. 497.
[20] Ztschr. f. Immunitätsf., 1913, 17, p. 548.

a carefully prepared 5% sheep cell suspension. The sheep blood was obtained from an ordinary laboratory sheep. The following were the results of the experiments:

RESULTS

First Series.—Rabbit 37 (weight 1.7 kg.); on Nov. 12, 15, 18 and 22, the average complement titer was 0.2 c c (1:2 dilution). The erythrocytes numbered 4,740,000, the platelets 853,200 and the leukocytes 8,600. Nov. 23, 24 and 25 this animal received 3 injections of 3.4 c c benzene olive oil mixture. Nov. 28, the leukocytes numbered 440, and on Nov. 29, the platelets numbered 70,000. The complement titer remained as before at 0.2 c c. The animal succumbed on Nov. 29. The bonemarrow of this animal was bloody and edematous; there was marked engorgement of blood vessels with numerous hemorrhages and relatively few myeloid cells. Scattered giant cells were seen. The spleen showed marked engorgement of sinuses, numerous hemorrhages; the blood pigment was in places intracellular, in places extracellular; malpighian bodies in places were smaller than the average. The liver showed no appreciable abnormalities.

Rabbit 36, weight 1.8 kg., on Nov. 12, 15, 18, and 22, the animal had an average complement titer of 0.21 c c (1:2 dilution). The erythrocytes numbered 4,964,000, the platelets 397,120 and the leukocytes 11,120. Nov. 23, 24 and 25, three injections of 3.6 c c of benzene olive oil mixture were made. Nov. 27, the white count dropped to 540 cells per c.mm., the platelets numbered 616,000. Nov. 27 and 28, the complement titer was 0.2 c c (1:2 dilution). This animal died on Nov. 28. The bone marrow was also bloody, edematous; there was marked engorgement of blood vessels and relatively few myeloid cells present. In the spleen the sinuses were moderately engorged; the malpighian bodies were smaller, the trabeculae conspicuous. The liver was normal.

Rabbit 23 (weight 2.2 kg.), on Nov. 15, 18 and 22 had an average complement titer of 0.25 c c (1:2 dilution). The erythrocytes numbered 6,316,000, the platelets 536,800 and the leukocytes 8,740. Nov. 23, 24, 25 and 27, four injections each of 4.4 c c benzene olive oil mixture were made. On Nov. 30, the white count dropped to 1000, and on Dec. 1, it was 800. On Dec. 1, the platelets numbered 50,000. Throughout the experiment the complement titer of this animal remained 0.25 c c. The bonemarrow on section showed almost contiguous engorgement of blood vessels, in places the vessel walls ruptured; there were few myeloid cells present. The sinuses in the spleen were engorged with blood; there was much intercellular and extracellular altered pigment; the malpighian bodies were hardly recognizable and in places appeared smaller than the average. The liver showed areas of scarring, especially marked about the large vessels. In general, there was no abnormality of the parenchyma.

Rabbit 30; control; weight, 1.6 kg.; on Nov. 12, 15, 18 and 22, the complement titer averaged 0.21 c c (1:2 dilution). The erythrocytes numbered 6,264,000; the platelets 500,000 and the leukocytes 7,305. This animal received 3 injections of olive oil in doses of 1.6 c c on Nov. 23, 24, 25. On Nov. 28, the complement titer was 0.25 c c and on Dec. 1, also 0.25 c c. On Nov. 29, the white count was 13,280, and on Dec. 1, 8700. The platelet count on Dec. 1 was 700,000. The animal survived.

Rabbit 39; control; weight, 2.6 kg.; Nov. 22 and 23 the complement titer averaged 0.2 c c (1:2 dilution). The erythrocyte count was 5,076,000; the platelets numbered 500,000 and the white cells 9,720. Nov. 23, 24 and 25, this animal received each day 2.6 c c of olive oil. On Nov. 28 and Dec. 1, the

complement titer remained at 0.2 c c; the platelets, respectively, 800,000 and 900,000; and the white cells fell from 15,560, on Nov. 28 to 11,240, on Dec. 1. The animal survived.

Rabbit 10; control; weight, 1.6 kg.; on Nov. 12, 15, 18 and 22, the complement titer was 0.2 c c (1:2 dilution). The erythrocytes numbered 5,644,000, the platelets 400,000 and the white corpuscles 11,895. This animal received no injections. On Nov. 28, 29 and Dec. 1, the complement titer remained as before. On Nov. 28, the red cells numbered 5,944,000; the platelets 540,000; and the white cells 7,680.

Second Series.—In this series the same experiments were repeated. The only difference between the first and second series was the method of platelet count as indicated in the foregoing.

Rabbit 290; weight, 1.8 kg.; on April 12, 13 and 14, the complement titer of this animal averaged 0.23 c c (1:2 dilution), the platelets 784,000, the white cells 15,200. The rabbit received 3.6 c c benzene olive oil mixture daily on April 15, 16, 17 and 18. On April 19, the white count dropped to 1400; the platelets were 752,000. The complement on April 19 was 0.2 c c and April 20, 0.15 c c. The animal died on April 23. The pathologic picture was the same as in the other rabbits that died of the poison.

Rabbit 293; weight, 1.6 kg; April 12, 14 and 15, the complement titer of this animal was 0.18 c c (1:2 dilution). The platelets numbered 636,000 and the whites 17,770. On April 15, 16, 17 and 18 the animal received 4 doses each of 3.2 c c benzene olive oil mixture. The white count dropped on April 19 to 500; the platelets, however, were slightly higher, namely, 754,000. On April 20, the platelets were 664,000; the white cells 580. The complement on April 19 was 0.2, and on April 21, 0.15 c c. On this day the animal died, and the postmortem picture was the same as in the other animals.

Rabbit 294; control; weight, 1.6 kg.; on April 12, 14 and 15 the complement of this animal titered 0.25 c c (1:2 dilution); the platelets numbered 368.000; the white count was 17,560. On April 15, 16, 17 and 18, the animal received 4 injections of 1.6 c c olive oil. On April 19 and 21, the complement titer did not change. On April 19, the platelets numbered 416,000 and the leukocytes 24,000, which count dropped to 9,000 April 20. The animal remained normal.

SUMMARY

From these experiments it appears that the rabbit readily responds to the action of benzene. In every instance the leukocytes were brought down to a figure below 1,000. The same is not true for the platelets. Some animals had a slight increase in platelets, an observation already made by Duke.[21] The control animals that received olive oil alone showed a transient leukocytosis. The complement titer of all the animals remained within normal variations so that it is fair to conclude that the reduction of the leukocytes and platelets in the circulating bloodstream does not go parallel with a change in the complement titer of the blood serum of the treated animals. Since the bone-marrow and spleen exhibit serious deterioration as the result of the benzene treatment, it is apparent that these tissues are not concerned in

[21] Jour. Am. Med. Assn., 1915, 65, p. 1600.

any important way in the maintenance of the complement of the serum. Whether the diminution of leukocytes is only apparent, or, as suggested by Pappenheim,[22] they accumulate in the dilated capillaries of the liver and other organs is, at present, difficult to state. Our observations indicate that they do not accumulate in the liver. The condition of the marrow strongly suggests that there is an actual reduction in the number of circulating leukocytes, and most modern studies point toward an absolute reduction. Antibodies are reduced following benzene injection, as pointed out by Hektoen.[23] On the contrary, the complementing power of the blood serum is not altered as the result of subcutaneous injection of benzene.

[22] Wien. klin. Wchnschr., 1913, 26, p. 48.
[23] Jour. Infect. Dis., 1916, 19, p. 69.